IN THE U.S. PATENT & TRADEMARK OFFICE

Applicants: Shozo KOYAMA et al.

Serial No.: 10/786,369 Group: 1641

Filed: Feb 26, 2004 Examiner: Shafiqul Haq

For: METHOD FOR PRODUCING AN ANTIGENIC SUBSTANCE AND ANTIBODY

DECLARATION UNDER 37 C.F.R. § 1.132

Honorable Commissioner of Patents and Trademarks

Washington, D.C., 20231

Sir:

I, Shozo KOYAMA, a nation of Japan, residing at 48-2, Oazasatoyamabe, Matsumoto-shi, Nagano 390-02, Japan, do hereby declare as follows:

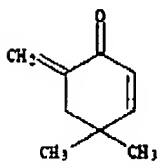
I am a co-applicant of the invention as described and claimed in the specification of the above-identified application.

I am familiar with the Office Action dated October 08, 2008, in which claim 35 is rejected as failing to comply with the written description requirement.

To show that the compound of Formula 3-a is surely effective for treating cancer other than leukemia and melanoma, I carried out the experiments described below.

Experiments

The compound of Formula 3-a, 4,4-dimethyl-6-methylene-2-cyclohexen-1-one (hereinbelow referred to as "Yoshixol"), was synthesized as shown in the specification and tested for its effect.



(1) Preparation of Vaccine against Murine Mesothelioma

Mouse mesothelioma cells (the cell line the Applicants established) were incubated in 2 ml of culture medium (Minimum Essential Medium, Gibco Co.; glutamine, Dai-Nipon Pharmace. Co.) supplemented with fetal bovine serum (Dai-Nipon Pharmace. Co.) under 5% CO_2 in an incubator at 37°C for 30 hours. When the number of cells reached about 2×10^5, cell death was induced by adding 4 µl of 2 M Yoshixol solution in ethanol. After confirming extinction of cells in culture medium, the culture medium containing extincted cells was centrifuged at 1,000 rpm for 5 minutes and the supernatant was removed by aspiration. To the sediment, 0.9 cc of physiological saline was added, and the mixture was stirred and centrifuged at 1,000 rpm for 5 minutes, followed by removing the supernatant by aspiration. This procedure was repeated twice. To the rinsed sediment, 0.9 cc of physiological saline was added, and the resulting mixture was stirred, followed by filtrating the mixutre through Millipore-filter of cellulose acetate having 0.45 µm pore to obtain a vaccine against mesothelioma.

(2) Effect of Vaccine

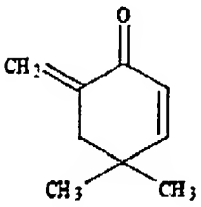
The filtered solution obtained in (1) (i.e., a vaccine against mesothelioma) was injected intraperitoneally into C57/BL mice (female, 6 weeks old, purchased from Nihon SLC Co.) in an amount of 0.2 cc per a mouse. Thirty days after the vaccination, 0.3 cc of culture medium of mouse mesothelioma cells (2×10^5/ml) which had been cultured independently from the above-described culture was transplanted to the back of vaccinated mice and control mice (without vaccination), respectively. The growth of mesothelioma cells in each mouse was measured 45 days after the transplantation. The result are shown in Figure 1.

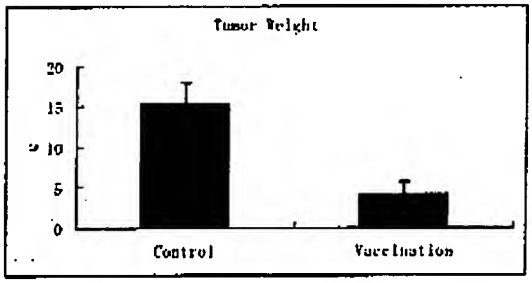
As shown in Figure 1 (top), the average amount of tumor in control mice reached more than 15 g. In contrast, an average amount of tumor in vaccinated mice was less than 5 g.

Figure 1 (middle and bottom) shows the appearance of vaccinated mouse and control mouse (45 days after the transplantation).

Murine Mesothelioma

Yoshixol





Control

Vaccination

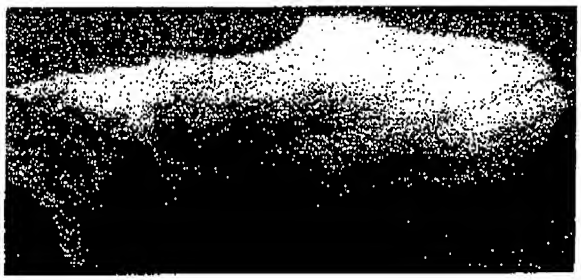


Figure 1

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 2nd day of January, 2009

Shozo KOYAMA